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Effectiveness of indoxacarb and hexaflumuron on eggs, larvae and adults of *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae)

Mohammad MAHMOUNDVAND[1,4], Habib ABBASIPOUR[1,*],
Aziz Sheikhi GARJAN[2], Ali Reza BANDANI[3]

(1. Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran; 2. Iranian Research Institute of Plant Protection, Tehran, Iran; 3. Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran; 4. Islamic Azad University, Khorramabad Branch, Khorramabad, Iran)

Abstract: Chemical control is the mainly control method for *Plutella xylostella* (L.) throughout the world. Choosing insecticides with high efficacy on all stages of this pest can help us control it well. In this case, toxicities of indoxacarb and hexaflumuron on eggs, larvae and adults of *P. xylostella* were studied with leaf dip method. The LC_{50} values of egg stage for indoxacarb and hexaflumuron were 201.40 and 37.32 mg/L, respectively. Also indoxacarb and hexaflumuron had excellent toxicity on the 3rd larval instar (LC_{50}: indoxacarb = 4.82 mg/L, hexaflumuron = 1.48 mg/L). Results of moth toxicity indicated that above insecticides had no acceptable toxicity on this insect (LC_{50}: indoxacarb = 845.20 mg/L, hexaflumuron = 3 438 mg/L). According to the calculated LC_{50} values, hexaflumuron was more toxic than indoxacarb to eggs and larvae of diamondback moth but at the adult stage the indoxacarb was more toxic.

Key words: Diamondback moth; toxicity; indoxacarb; hexaflumuron; adulticide; larvicide; ovicide

1 INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lep.: Yponomeutidae), is a serious pest of crucifers in Iran. It feeds on broccoli, cabbage, chinese cabbage, cauliflower, kale, mustard, radish and turnip in the world (Talekar and Shelton, 1993; Capinera, 2001; Mahmoudvand *et al.*, 2009). Females of diamondback moth may deposit 250 - 300 eggs in a year (Capinera, 2001). Though *P. xylostella* has expanded resistance to different classes of insecticides, the chemical control is applied as conventional control method against it (Sun *et al.*, 1986; Shelton *et al.*, 1991). An oxadiazine insecticide, indoxacarb is effective against a wide spectrum of pests by ingestion and contact. In addition it has ovicidal effect. Indoxacarb is a good choice for controlling Lepidoptera pests and have shown low side effect on the non-target insects (Harder *et al.*, 1996; Wing *et al.*, 2000; Sparks *et al.*, 2001; Burkness *et al.*, 2003; Michaud and Grant, 2003; Boiteau and Noronha, 2007). The action mode of indoxacarb is to block the sodium channels of nerve cells (Meier *et al.*, 1992). Hexaflumuron as an insect growth regulator (IGR) is safer than other insecticide classes. This insecticide class affects endocrine systems, interrupts molting (juvenile hormone) and disturbs cuticle formation (chitin synthesis inhibitors) (Dhadialla *et al.*, 1998; Pedigo and Rice, 2002). Chitin synthesis inhibitors are a group of IGRs that disrupt the development of insect life cycle (Verloop and Ferrell, 1977). A group of these, benzoylphenyl urea (BPU), can be used in IPM programs because of their selectivity properties (Wright and Retnakaran, 1987). Hexaflumuron is a BPU insecticide that inhibits chitin synthesis and interrupts the process of molting in targets. However the main toxicity of hexaflumuron is through ingesting but also it has contact and ovicidal toxicity (Sbragia *et al.*, 1983; El-Barkey *et al.*, 2009).

Efficacy on various stages of insect pest is an important and economical factor for choosing an insecticide. The diamondback moth has become a serious threat for crucifers in Tehran province and so far no insecticides have been registered in Iran. Unfortunately chemical control studies on diamondback

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* Corresponding author, Tel.: +98-21-51212261; Fax: +98-21-51212251; E-mail: habbasipour@yahoo.com

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moth were focused only on larval stage that is destructive stage of this pest, whereas the adult stage for producing next generation is very important. To the present authors' knowledge there are no existing studies regarding the toxicity of hexaflumuron on egg and adult stages of diamondback moth. Hence in this study, we tested the toxicity of indoxacarb and hexaflumuron on various stages (eggs, 3rd larval instars and adult moths) of diamondback moth and estimated the lethal concentration (LC_{50}) values of these insecticides on various stages.

2 MATERIALS AND METHODS

2.1 Insecticides

The formulated insecticides in these experiments were indoxacarb [Avaunt®, 150 SC (AI), DuPont Company, Cernay, France] and hexaflumuron [Consult®, 10% EC (AI), DowAgrosciences Company].

2.2 Insect rearing

Initial *P. xylostella* population was collected from cauliflower crops at Shahre-Rey in south of Tehran, Iran. For egg laying, about 500 adults of *P. xylostella* were released in a plastic cage (50 cm × 30 cm × 30 cm) and eggs were transferred to cauliflower leaves, *Brassica oleracea* (Brassicaceae) to continue their development. Insect stock was maintained at 25 ± 1℃ and 65% ± 5% relative humidity under 16∶8 (L∶D) cycle in a growth chamber.

2.3 Ovicidal effect assay

For all bioassay experiments, a leaf dip method was used (Tabashnik and Cushing, 1987). Cabbage leaf disks containing 10 eggs (< 10 h old) of *P. xylostella* (leaves and eggs together) were dipped in different concentrations of indoxacarb and hexaflumuron solutions including 0.02% Tween-80 for 30 s. For control, the leaf disks were dipped in water with 0.02% Tween-80. The treated leaf disks were permitted to dry at room temperature for 2 h. Then the leaf disks were placed in Petri dishes (8 cm in diameter, 1.5 cm deep). The eggs were checked daily until all larvae hatched or the eggs died. Each treatment had 4 replications and at least 60 eggs were used for each concentration.

2.4 Bioassay of larval stage

Cabbage leaf disks (3 cm in diameter) were dipped in different concentrations of above insecticide solutions containing 0.02% Tween-80 for 30 s. In controls, the leaf disks were dipped in water and 0.02% Tween-80. After the treated leaves were dried, they were placed in a plastic cup (3 cm deep and 5.5 cm in diameter). Ten one day old-third instar larvae, which were starved for 2 h, were placed on the leaf disks. These tests were repeated 4 times and at least 60 3rd instar larvae were used for each concentration. Mortality was recorded 48 and 96 h after treatment for indoxacarb and hexaflumuron, respectively.

2.5 Bioassay of adult stage

For adult bioassay, leaf disks (8 cm diameter) were cut and dipped for 30 s in several concentrations of mentioned insecticide solutions. For control, water with 0.02% Tween-80 was used. After the leaves were dried, they were placed on the bottom of plastic Petri dishes (8 cm in diameter, 1.5 cm deep). Then, ten individual moths (<24 h old) were placed on the leaf disks in each Petri dish. A cotton piece contain sugar solution (10%) was used to feed the moth adults at these tests. These tests were repeated 4 times and at least 70 moths were used for each concentration. Mortality was recorded 24 h after treatment.

2.6 Data statistics and analysis

The data were analyzed in Probit analysis using SAS software (SAS Institute, 1997). For comparing the toxicity of the insecticides, overlapping and toxicity ratio (TR) indexes based on 95% confidence limits were used (Robertson *et al.*, 1992).

3 RESULTS

3.1 Ovicidal effect

Table 1 shows LC_{50} values of tested insecticides on eggs of diamondback moth. The results showed that both indoxacarb and hexaflumuron, had good activity on egg hatching of *P. xylostella*. The estimated LC_{50} value of indoxacarb (201.40 mg/L) was higher than that of hexaflumuron (37.32 mg/L). There was a significant difference between ovicidal effect of indoxacarb and hexaflumuron. Toxicity of hexaflumuron was 5.39 times higher than indoxacarb. Thus both insecticides have ovicidal.

3.2 Larvicidal effect

Indoxacarb and hexaflumuron had acceptable effects on 3rd larval instar of diamondback moth (Table 1). In this study, larvicidal activity was based on ingestion toxin of these insecticides. The calculated LC_{50} values of indoxacarb and hexaflumuron were 4.82 and 1.48 mg/L, respectively. Larvicidal property of hexaflumuron as well as its ovicidal was significantly higher than indoxacarb. Results showed that larvicidal effect of hexaflumuron was 3.25 fold of indoxacarb. Larvicidal effect of indoxacarb was

41.87 fold higher than its ovicidal. Also larvicidal effect of hexaflumuron was 25.21 fold higher compared with its ovicidal.

3.3 Adulticidal effect

In general, the LC_{50} values for adult moths were much higher compared with those for eggs and larvae (Table 1). LC_{50} values of adult bioassay for indoxacarb and hexalumuron were counted 845.20 and 3 438 mg/L, respectively. Contact toxicity of indoxacarb on adult stage of the diamondback moth was 4.06 fold higher than hexaflumuron. Ovicidal and larvicidal efficacies of indoxacarb and hexaflumuron were severely higher than their adulticidal efficacy on the diamondback moth.

Table 1 Results of leaf dip bioassay of indoxacarb and hexaflumuron on eggs, 3rd instar larvae and adults of *Plutella xylostella*

Developmental stage of test insect	Test insecticide	n[a]	*df*	LC_{50} (mg/L)	Slope ± *SE*	Intercept ± *SE*	χ^2	TR
Egg	Indoxacarb	434	3	201.40(181.93 - 226.81)	4.25 ± 0.55	- 9.81 ± 1.25	1.41	5.39
	Hexaflumuron	377	4	37.32(25.58 - 57.69)	1.07 ± 0.22	- 1.68 ± 0.34	4.44	1
3rd larva	Indoxacarb	390	4	4.82(3.59 - 6.23)	1.48 ± 0.20	- 1.01 ± 0.18	2.47	3.25
	Hexaflumuron	453	5	1.48(1.28 - 1.67)	3.20 ± 0.44	- 0.55 ± 0.13	2.57	1
Adult	Indoxacarb	390	3	845.20(769.41 - 922.39)	4.42 ± 0.82	- 12.94 ± 2.42	1.06	1
	Hexaflumuron	430	4	3 438(3 337 - 3 534)	13.04 ± 1.68	- 46.14 ± 5.98	3.09	4.06

[a] Number of subjects. TR: Toxicity ratic.

4 DISCUSSION

Results of this study indicated that indoxacarb and hexaflumuron had ingestion and contact effects on *P. xylostella*. Also these insecticides have shown ovicidal effectiveness. Whereas all of these tests were experimented through leaf dip method, the effects of insecticides in each stage were different. Insecticides in ovicidal test inhibit egg hatch at seepage outcome. This is the first study in ovicidal effect of hexaflumuron on *P. xylostella*. In contrast of our results, Liu *et al.* (2003) reported that indoxacarb has no significant effect on eggs of *P. xylostella*. Liu *et al.* (2002) evaluated the effects of indoxacarb on eggs and larvae of *Trichoplusia ni* (Hübner) (Lep.: Noctuidae) and observed that LC_{50} value of egg stage is higher than that of larval stage. Results of this study, in ovicidal and larvicidal experiment relatively supported results of Liu *et al.* (2002). El-Barkey *et al.* (2009) reported that hexaflumuron had ovicidal effect on a lepidopteran pest, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). Oouchi (2005) reported that an IGR, pyriproxyfen at 100 mg/L concentration caused egg mortality of *P. xylostella* up to 89%. In this study, hexaflumuron had higher ovicidal effect than indoxacarb. It may be due to its compounds that remove eggs cortex.

In larvicidal bioassay ingestion effects of insecticides were evaluated and it was higher for hexaflumuron than indoxacarb. Results obtained from this bioassay indicated that indoxacarb and hexaflumuron are both toxic to *P. xylostella*. Eziah *et al.* (2008) investigated the effects of indoxacarb on one susceptible and four unsusceptible stocks of *P. xylostella*. The LC_{50} values were 0.068, 0.774, 1.97, 2.42 and 1.711 μg/larva, respectively. Nehare *et al.* (2010) tested indoxacarb on a number of generations of *P. xylostella* and the LC_{50} values were estimated 18.5 and 167.8 mg/L for the 1st and 10th generations, respectively. Kobayashi *et al.* (1990) investigated the effect of hexaflumuron on larvae of diamondback moth. In that study, mortality was recorded after 5 days. The calculated lethal concentration (LC_{50}) values of hexaflumuron in their research were 1.8 and 2.53 mg/L in unsusceptible strain of the diamondback moth. In this study, the LC_{50} value of hexaflumuron is almost similar to that in research of Kobayashi *et al.* (1990) strain, although in the current study, feeding time was 96 h. The difference between the present LC_{50} values and mentioned studies can be due to the different biotypes of the insects.

There are few studies regarding effect of insecticides on adult stage of lepidopteran pests and most reports focus on destroyer stage control, thus knowledge about the effect of pesticide residues on adults of *P. xylostella* is so little (Idris and Grafius, 1993). To the best of our knowledge, there is no study about effect of indoxacarb and hexaflumuron on adults of the diamondback moth. Ruscoe (1977) found that toxicity of permethrin residues on cabbage leaves on larvae of *P. xylostella* were more toxic than adults. Our results indicated that the efficacy of indoxacarb and hexaflumuron on the diamondback moth at adult stage was less than that at its larval and egg stages. This severe difference is maybe the methods of getting toxin, because moths get toxin only by contact. Hill and Foster (2000) reported that in treated moths of the diamondback moth with spinosad, tebufenozide and permethrin, high mortalities were observed. Isaacs *et al.* (2005) stated that adult mortality of grape berry moth, *Endopiza viteana* (Clemens) (Lepidoptera: Tortricidae) was not affected by exposure to field-equivalent rates of tebufenozide and methoxyfenozide on grape clusters. Unlike this study, LC_{50} values in study of Hill and Foster (2000) were low, while time of recording was different.

In conclusion, results of this study indicated that indoxacarb and hexaflumuron are highly toxic to *P. xylostella* larvae with low LC_{50} values. But, according to our results, these insecticides have more acceptable ovicidal effect compared with its adulticidal activity.

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茚虫威和六伏隆对小菜蛾卵、幼虫和成虫的药效

Mohammad MAHMOUNDVAND[1,4], **Habib ABBASIPOUR**[2,*], **Aziz Sheikhi GARJAN**[2], **Ali Reza BANDANI**[3]

(1. Department of Plant Protection, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran; 2. Iranian Research Institute of Plant Protection, Tehran, Iran; 3. Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran; 4. Islamic Azad University, Khorramabad Branch, Khorramabad, Iran)

摘要： 化学防治是世界各地防治小菜蛾 *Plutella xylostella* (L.)的主要方法。选择对小菜蛾各发育阶段高效的杀虫剂有助于很好地控制这一害虫。本研究用浸叶法测定了茚虫威和六伏隆对小菜蛾卵、幼虫和成虫的毒性。茚虫威和六伏隆对小菜蛾卵的 LC_{50}值分别为 201.40 和 37.32 mg/L。茚虫威和六伏隆对3龄幼虫也有很好的毒性(茚虫威的 LC_{50}为 4.82 mg/L；六伏隆的 LC_{50}为 1.48 mg/L)。对成虫的毒性结果表明，这两种杀虫剂对成虫均没有理想的毒性(茚虫威的 LC_{50}为 845.20 mg/L；六伏隆的 LC_{50}为 3 438 mg/L)。根据计算的 LC_{50}值，六伏隆对小菜蛾卵和幼虫的毒性比茚虫威高，但是茚虫威对成虫的毒性更高。

关键词： 小菜蛾；毒性；茚虫威；六伏隆；杀成虫剂；杀幼虫剂；杀卵剂

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